IX. "On the Disappearance of the Leucocytes from the Blood, after Injection of Peptone." By Surgeon-Captain DAVID BRUCE, A.M.S., Army Medical School, Netley. Communicated by Professor HORSLEY, F.R.S. Received February 21, 1894.

As is well known, the injection of a solution of peptone into the circulation of certain animals is followed immediately by a very considerable diminution in the number of white blood corpuscles in the circulating blood. Some investigators who have written on this subject ascribe this diminution to the destruction and breaking down of the leucocytes in the blood plasma.

As this theory appeared to me to rest on the very slenderest evidence, and as it seemed to me much more natural to believe that a temporary withdrawal of the leucocytes into the internal organs took place, I was led to attempt the enumeration of the white blood corpuscles in sections of the various organs before and after the injection of peptone.

If the theory of destruction is true, then the leucocytes ought to be found in fewer numbers in the organs as well as in the blood, whereas, if the theory of temporary withdrawal be the true one, then an augmentation in their number should be seen in the sections.

For the purpose of this enumeration I used rabbits of equal weight, and proved to have a normal number of white blood corpuscles by examination of samples of blood taken from the ear.

Six rabbits were taken. The organs of two of these were examined without previous injection of peptone. The third, with a normal number of white blood corpuscles, was killed $3\frac{1}{2}$ hours after the injection of the peptone solution, when $\frac{4}{5}$-ths of the white blood corpuscles had disappeared from the circulating blood.

The fourth, also having a normal number of white blood corpuscles, was killed 5 seconds after injection, when almost all the leucocytes had disappeared, at least from the blood of the heart.

In the fifth and sixth a leucocytosis was first caused by the injection of appropriate fluids and the animals killed 5 seconds after the injection of peptone.

As it seemed to me impossible to be certain of recognising all the varieties of the leucocytes in sections of the organs, I restricted myself to enumerating what I know as the polynuclear variety, since this is the variety which disappears most completely from the blood after the injection of peptone and many other substances, and which can be very readily recognised in sections after appropriate staining.

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I may mention here that for practical purposes and without prejudice as to their origin, I adopt the classification of the leucocytes of the rabbit's blood into four varieties :—

A. The Eosinophilous, constituting on an average 2 per cent. of the total leucocytes, with an irregular-shaped nucleus and large oval shaped granules, which stain readily in eosine.

B. The Polynuclear, 51 per cent., with intensely staining polymorphous nucleus, having the appearance of several nuclei united by narrow thread-like processes, the protoplasm of the cell containing fine granules which also stain in eosine.

C. The Myelocytes, 16 per cent. Difficult in every case to rigidly separate from the fourth variety, but defined as mononuclear cells, the nucleus of which stains badly, and is surrounded by comparatively a large amount of non-granular protoplasm.

D. The Lymphocytes, 31 per cent. Mononuclear cells, little larger than a red blood corpuscle, with intensely staining nucleus and narrow rim of protoplasm.

The preliminary enumeration of the leucocytes in the blood of the ear or heart was made by diluting the blood 200 times with an 8 per cent. magnesium sulphate solution, to which sufficient gentian violet had been added to stain the white blood corpuscles, and not by estimating their proportion to the red blood corpuscles, which seems to me a most unsound method. The number of white blood corpuscles in 300 squares of a Gower's hæmocytometer were then counted and this number multiplied by 333, which gives approximately the number in a cubic millimetre.

Three samples of blood are taken, two of which are counted, and, if the counts are sufficiently close, an average made. In the event of there being a marked discrepancy, the third sample is counted and an average of the two most alike taken, but, with care, it is seldom found necessary to use the third sample.

In regard to the fixing and hardening of the tissues for cutting, alcohol, Müller's solution, Foa's, and Flemming's solutions were used, but the best results were obtained by fixing for twenty-four hours in Flemming's strong solution, washing in running water for twenty-four hours, then through successive alcohols for forty-eight hours.

The tissues, after infiltration with paraffin, were cut as neatly as possible of the same thickness, and stained in various ways, the most successful results being got by staining for twenty-four hours in the Ehrlich-Biondi triple stain.

On taking the sections out of the stain I place them for a minute in a saturated solution of corrosive sublimate, as this seems in some way to prevent them becoming too much decolorised during the subsequent manipulations.

The white blood corpuscles contained in twenty fields of the micro-

scope (Zeiss' 2 millimetres apochromatic, oil immersion, and 8 eye-piece) were counted in each section, several of which were mounted from each organ, and, as will be seen, with very striking uniformity of results.

I shall now proceed to describe the results obtained in each experiment.

No. 1. *Control Experiment.*—Rabbit, weight 2 kilos. The blood from an ear-vein was found to contain 6500 leucocytes per cubic millimetre—0 per cent. eosinophilous, 34 per cent. polynuclear, 20 per cent. myelocytes, 46 per cent. lymphocytes—of which 2210 were thus polynuclear. The animal was now killed by a blow on the head and the organs at once removed. Four sections of the spleen were found to contain 54, 47, 55, and 54 polynuclear leucocytes respectively (twenty fields of the microscope being counted in each specimen), the lung, 39 and 32, and the liver, 7, 6, and 7.

It will be seen from the above enumerations that a remarkable uniformity was found to exist in the distribution of the polynuclear leucocytes in different sections of the same organ. This, I may here mention, obtained throughout, not only in the control experiments, but also after the injection of peptone.

No. 2. *Control Experiment.*—Rabbit, 2 kilos. The blood from an ear-vein was found to contain 3833 polynuclear leucocytes in each cubic millimetre. Two sections of the spleen were counted and contained, in 20 fields, 62 and 74 respectively; the lung, 35 and 41; liver, 5 and 6.

No. 3. *Control Experiment.*—To show that the blood contained in the right and left ventricles does not differ markedly from the blood taken from an ear-vein in the number of leucocytes contained in the cubic millimetre the following experiment was made:—

Rabbit, 2 kilos. The blood from an ear-vein was found to contain 8500 leucocytes in each cubic millimetre. After an hour the rabbit was killed by a blow on the head, and the heart ligatured and removed as soon after death as possible.

The blood of the right ventricle was found to contain 7200 leucocytes per cubic millimetre; that of the left, 7500.

The following experiment shows the number of polynuclear leucocytes found in sections of the organs of an ordinary normal rabbit after the leucocytes have been greatly diminished in the circulating blood by the injection of peptone.

No. 4. Rabbit, 2 kilos. Blood from the right ear contained 4015 polynuclear leucocytes. Three and a half hours after the injection into a vein of the left ear of 3·5 grams peptone, dissolved in 35 c.c. water, blood from the right ear only contained 1100 of the same variety.

Sections of the spleen prepared and examined in the same way as

in Experiments 1 and 2, contained 159, 150, 171, and 168 polynuclear leucocytes in 20 fields of the four specimens examined; the lung, 230 and 209; the liver, 38 and 35 respectively.

This experiment then shows a large increase in the number of the polynuclear leucocytes in the organs, as compared with what was found in the organs of the control animals.

But, as the injection of peptone causes not only a primary diminution but also a secondary augmentation in the number of leucocytes in the blood, it might be argued that this augmentation takes place at first in the organs, and that this might account for the increase found in Experiment No. 4.

To meet this objection the following experiment was made :—

No. 5. Rabbit, 2 kilos. Blood from ear contained 9200 leucocytes, of which 47 per cent. or 4424, belonged to the polynuclear variety; 20 c.c. of a 10 per cent. peptone solution were now rapidly injected into the left jugular vein, and the animal killed five seconds after the injection.

The blood contained in the heart was at once examined, that in the left ventricle being found not to contain a single leucocyte of any kind whatever; that of the right only 166 per cubic millimetre, all told. It must be noted, however, that the leucocytes do not disappear from all parts of the circulation with this marvellous rapidity, as Experiment No. 7 will show.

The organs in this case were also found to contain a great excess of polynuclear leucocytes: the spleen, 174, 133, and 149; the lung, 230 and 221; the liver, 12 and 14.

I next tried the effect of first causing a leucocytosis, and then driving the white blood corpuscles out of the blood by peptone, in the belief that some proportionate increase in the number of the polynuclear leucocytes in the organs might be found.

No. 6. Rabbit, weight 2 kilos. Blood from ear contained 7800 leucocytes, of which 4290 belonged to the polynuclear variety.

Nineteen hours after the injection of 10 c.c. of a broth cultivation of anthrax, which had been sterilised by filtration through a Chamberland filter, the number of leucocytes in the blood taken from the ear had increased to 17,600, of which 11,264 were polynuclear. 20 c.c. of a 10 per cent. peptone solution were now injected into the jugular vein, and, five seconds afterwards, the animal killed. Blood from the left ventricle contained no leucocytes; that from the right ventricle only 1500 per cubic millimetre.

The spleen sections contained 223 and 218, the lung 251 and 247, the liver 12 and 10.

Experiment 7. Rabbit, 2 kilos. The blood taken from an ear-vein contained 14,300 white-blood corpuscles.

20 c.c. of a 10 per cent. peptone solution were injected into an

ear-vein, and, five seconds afterwards, the white-blood corpuscles in the opposite ear enumerated, when no diminution in their number was found. Eighteen hours after the injection an intense leucocytosis was found to have become developed, no fewer than 184,500 white blood corpuscles being found in each cubic millimetre of blood drawn from the ear.

Again, 20 c.c. of a 10 per cent. peptone solution were injected; this time by the left jugular vein, and, five seconds afterwards, the animal killed. The blood in the left ventricle contained 1000 white blood corpuscles per cubic millimetre, the right ventricle 3000, and the inferior vena cava in the lumbar region 5000.

Sections of the spleen contained 262 and 308, the lung 435, 325, and 348, and the liver 78 and 82.

Presenting, for the sake of clearness, the above facts in a tabular form, an average of the various numbers being set down, the contrast is striking.

Organs examined.	Control experiment.	Control experiment.	Normal rabbit, 3½ hours after injection of peptone.	Normal rabbit, 5 seconds after injection of peptone.	Rabbit with slight leucocytosis after injection of peptone.	Rabbit with intense leucocytosis after injection of peptone.
Spleen.....	52	78	160	152	220	235
Lung.....	39	38	214	225	249	369
Liver.....	6	5	36	13	11	80

From the above table it is seen that the lung harbours a greater proportion of the leucocytes which have disappeared from the blood than any other organ. This may be accounted for by the fact that this organ is the first acted on by the peptone solution after its injection into the jugular vein.

Other organs, such as the kidney, supra-renals, and ovary, were found to contain so few leucocytes, either before or after the injection of peptone, that the results as regards them have been neglected.

I conclude from the above experiments that the injection of a solution of peptone into the circulation of rabbits does not cause, as has been asserted, a destruction of leucocytes, but merely a withdrawal of them into various organs, notably the lungs and spleen.

The Society then adjourned over the Easter Recess to Thursday, April 19.

Observations and Reports.

Calcutta:—Meteorological Department, Government of India. Meteorological Observations recorded at Seven Stations in India. September, 1893. 4to.; Monthly Weather Review. September, 1893. 4to. *Calcutta* 1894. The Department.

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Annaes de Sciencias Naturaes. Anno. I. No. 1. 8vo. *Porto* 1894. The Editor.

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Technology Quarterly. Vol. VI. No. 3. 8vo. *Boston* 1893. Massachusetts Institute of Technology.

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Schück (A.) Magnetische Beobachtungen an der Unterelbe, angestellt im Jahre 1893. 8vo. *Hamburg* 1894. The Author.

Vogel (H. C.) Über das Spectrum von β Lyrae. 8vo. *Berlin* 1894. The Author.

Photograph of the Indenture of Sir H. Davy, in the possession of the Royal Institution of Cornwall. Mr. John D. Enys.

Account of the appropriation of the sum of £4,000 (the Government Grant) annually voted by Parliament to the Royal Society, to be employed in aiding the Advancement of Science (continued from vol. liii, p. 321).

April 1, 1893, to March 31, 1894.

General Fund.

	£	*s.*	*d.*
A. M. W. Downing, for the Expense of Computations for a New Edition of Taylor's Madras Catalogue of Stars	100	0	0
Carried forward	£100	0	0

	£	s.	d.
Brought forward	100	0	0
W. G. Rhodes, for a Minute Study of the Electrical Temperature Coefficient of some Pure Metals, with the view of obtaining an Electrical Thermometer more reliable than the Platinum Thermometer	35	0	0
W. S. Wilson, to Make and Experiment with an Instrument which is designed to find the Direct Intensity of Solar Radiation	30	0	0
Dr. S. Young, for the Determination of the Vapour Pressures and Molecular Volumes, both as Liquid and Saturated Vapour, of various Substances, from Low Temperatures to their Critical Points	40	0	0
Dr. Edridge Green, for Continuation of his Experiments on Colour-blindness	60	0	0
Prof. C. V. Boys, for a Research on the Constant of Gravitation γ by a very Exact Method depending on the use of Quartz Fibres	50	0	0
G. Higgs, for Completing and Printing a Photographic Atlas of the Normal Solar Spectrum.....	50	0	0
Dr. Schuster, for Assistance in Certain Numerical Calculations concerning Terrestrial Magnetism	50	0	0
E. H. Griffiths, for (1) a Comparison of Air, Mercury, and Platinum Determinations of Temperature; (2) Continuation of Investigation into the Capacity for Heat of Water at Different Temperatures.....	60	0	0
A. Mallock, for the Measurement of the Constant of Viscosity of Fluids, and the Examination of the Conditions which lead to Instability in the Flow of Fluids past Solid Obstacles	50	0	0
A. J. Herbertson, for an Investigation into the Hygrometry of the Atmosphere at Different Altitudes	100	0	0
C. T. Heycock and F. H. Neville, for Continuation of Experiments on Solutions of Metals in Metals.....	70	0	0
J. Walker, for an Investigation of the Affinity of Weak Acids and Bases.....	20	0	0
S. U. Pickering, for further Research on the Nature of Solutions	50	0	0
W. P. Bloxam, for an Examination of the Compounds formed by Ammonium with Sulphur.....	50	0	0
S. E. Linder and H. Picton, for an Investigation into the Connection between different Grades of Solution	20	0	0
Carried forward.....	£835	0	0

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Brought forward	835	0	0
W. H. Perkin, jun., for an Investigation into the Constitution of Camphoric and Allied Acid	75	0	0
Dr. Tilden, towards defraying Expenses incurred in a Systematic Examination of the Action of Nitrosyl Chloride upon Carbon Compounds.....	50	0	0
H. B. Baker, for the Full Investigation of the Influence of Moisture on Various Types of Chemical Actions	120	0	0
Dr. Japp, for an Investigation of the Reactions of Ketones, Diketones, and Allied Compounds.....	75	0	0
S. F. Dufton, for a Research on the Hydrazines of Quinoline	50	0	0
E. P. Perman, for Continuation of a Research on Vapour Density..... ..	20	0	0
W. A. Shenstone, for Continuing the Investigation of the Influence of Silent Discharge of Electricity on Oxygen and other Gases	30	0	
G. T. Moody, for the Study of Phenanthrene	50	0	0
F. S. Kipping, for the Preparation and Study of Cyclic Keto-derivatives, more especially those of Interest in relation to Camphor.....	60	0	0
The Pharmacological Committee (per Prof. Dunstan), for Pharmacological Investigations with Chemically Pure Substances of Ascertained Composition	200	0	0
J. T. Hewitt, for a Research on Ortho-substituted Phenyl-hydrazines.....	20	0	0
C. Davison, for the Investigation of British Earthquakes.....	25	0	0
J. H. Cooke, for an Investigation of the Nature, Distribution and Fauna of the Pleistocene Deposits of the Maltese Islands.....	50	0	0
Herbert Bolton, for an Investigation into the Age, Stratigraphy, and possible Fossil Contents of the Skiddaw Slates of the Isle of Man.....	20	0	0
R. Kidston, to Work out the Vertical and Horizontal Distribution of the British Carboniferous Flora	40	0	0
G. F. Scott-Elliot, for an Expedition to Investigate the Flora, Fauna, and Geology of Tanganyika, Uganda, &c.....	700	0	0
Bahamas Committee (per W. T. T. Dyer), for the Botanical Exploration of the Bahamas	150	0	0
Carried forward.....	£2,570	0	0

	£	*s.*	*d.*
Brought forward	2,570	0	0
West India Committee (per G. Murray), to continue the Work of the Joint Committee of the Royal Society and British Association for the Exploration of the Natural History of the West India Islands	100	0	0
Liverpool Marine Biology Committee (per W. Herdman), for the further Exploration of the British Marine Fauna, especially in the neighbourhood of Liverpool and the shores of the Isle of Man	50	0	0
E. J. Allen, for a Research on the Later Stages in the Development of Decapod Crustacea	200	0	0
Sandwich Islands Committee (per Dr. Sharp), for the Investigation of the Fauna of the Hawaiian Islands ..	200	0	0
Prof. J. R. Ainsworth Davis, for a Research on the "Locality Sense" in *Patella vulgata*	20	0	0
E. J. Bles, for further Research on the Marine Floating Organisms of the British Seas, and the effect of Changes of Environment on their Distribution.....	50	0	0
Dr. A. B. Harris, for the Investigation of Oxidation and Reduction Processes in Animals under various Pathological Conditions	20	0	0
W. M. Bayliss, on the Nature of the Action of the Vasomotor Centre.....	40	0	0
C. A. Ballance and S. Shattock, for a Research on the Intimate Pathology of Cancer.....	50	0	0
Dr. E. H. Starling, for a Research on the Physiology of Lymph Secretion	50	0	0
Dr. A. E. Garrod, for further Research on certain of the Urinary Pigments, and other Allied Investigations.....	20	0	0
Prof. P. F. Frankland, for Continuation of Researches on the Chemical Changes induced by Specific Micro-organisms	125	0	0
C. S. Sherrington, for the Examination of the Actions and the Topography of Reflex and Automatic Centres in the Lower Half of the Spinal Cord.....	50	0	0
W. G. Spencer, for a Research on the Central Innervation of the Respiration and Circulation in connexion with Increased Intra-cranial Pressure, &c.	75	0	0
Prof. W. D. Halliburton, for Researches on (1) Intravascular Coagulation (2) the Nutrition value of Hæmoglobin	50	0	0
Carried forward	£3,670	0	0

	£	*s.*	*d.*
Brought forward	3,670	0	0
Dr. Copeman, for further Aid in Researches on the Bacteriology of Vaccine Lymph	50	0	0
Joint Eclipse Committee, towards excess of Expenditure over Estimates.....	100	0	0
	£3,820	0	0

GENERAL FUND.

Dr.	£	*s.*	*d.*	*Cr.*	£	*s.*	*d.*
To Balance, March 31, 1893....	845	7	9	By Appropriations, as above.....	3,820	0	0
„ Parliamentary Grant	4,000	0	0	„ Salaries, Printing, Postage, Advertising, and other Administrative Expenses	108	7	0
„ Repayment.....	70	7	0	„ Transferred to Reserve Fund	500	0	0
„ Interest on Deposit.....	27	17	0	„ Balance, Mar. 31, 1894	515	4	9
	£4,943	11	9		£4,943	11	9

RESERVE FUND.

Dr.	£	*s.*	*d.*	*Cr.*	£	*s.*	*d.*
To Balance, Mar. 31, 1894.	900	0	0	By Balance, Mar. 31, 1894	1,400	0	0
„ Transfer from General Fund.....	500	0	0				
	£1,400	0	0		£1,400	0	0